REMARKS

Applicant respectfully requests reconsideration of the above-identified patent application in view of the amendment above and the remarks below.

No claims have been canceled or added herein. Claims 1 and 21 have been amended herein. Therefore, claims 1-24 are pending and are under active consideration.

Claims 1-5 and 7-23 stand rejected under 35 U.S.C. 103(a) "over Herman et al. (U.S. Patent 6,265,171 B1) (July 24, 2001) in view of Koster (U.S. Patent 5,605,798) (February 25, 1997)." In support of the rejection, the Patent Office contends, among other things, (i) that Herman et al. teaches steps (a) through (e) of the method of claim 1; and (ii) that Koster "teaches a method wherein the hybridized probes are analyzed in a mass spectrometer and the position of the probes on the sample holder permits a classification of the hybridizing DNA sample (Abstract, Examples 1-2, Figures 10-11, Column 4, lines 25-55, and claim 1)." The Patent Office then concludes by stating the following:

> It would have been prima facie obvious to one having ordinary skill in the art at the time the invention was made to combine and substitute the DNA diagnostic based on mass spectrometry of Koster in the method of detection of methylated nucleic acid using agents which modify unmethylated cytosine and distinguish modified methylated and unmethylated nucleic acids of Herman et al. since Koster states, "In addition, because the instant disclosed processes allow the nucleic acid fragments to be identified and detected at the same time by their specific molecular weights (an unambiguous physical standard), the disclosed processes are also much more accurate and reliable than currently available procedures (Column 4, lines 50-55)." An ordinary practitioner would have been motivated to combine and substitute the DNA diagnostic based on mass spectrometry of Koster in the method of detection of methylated nucleic acid using agents which modify unmethylated cytosine and distinguish modified methylated and unmethylated nucleic acids of Herman et al. in order to achieve the express advantages, as noted by

Koster, of processes which allow the nucleic acid fragments to be identified and detected at the same time by their specific molecular weights (an unambiguous physical standard), and which are also much more accurate and reliable than currently available procedures.

Later in the Office Action, in the section entitled "Response to Arguments," the Patent Office states the following:

> The request for reconsideration, filed on March 26, 2003, has been considered but does not place the application in condition for allowance because of the following reasons:
>
> A) Applicant argues (page 3, last paragraph to page 4, line 9) that Herman et al reference teaches only the amplification of methylated fragments and does not teach the amplification of both methylated and unmethylated fragments of the instant invention. This argument is not persuasive. In response to applicant's argument that the references fail to show certain features of applicant's invention, it is noted that the features upon which applicant relies (i.e., the amplification of both methylated and unmethylated fragments) are not recited in the rejected claim(s). Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See *In re Van Genus*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993).
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> Moreover, Herman reference clearly teaches the amplification of both methylated and unmethylated fragments (Column 23, line 67 to Column 24, line 3).
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> B) Applicant also argues (Page 4, last two sentences) that Herman reference does not teach the step(e) of removing any non hybridized probes from the immobilized DNA samples and there would be no reason to hybridize any probes to the MSP amplificates for the purpose of indicating methylation and there would be no reason to remove any non-hybridized probes. This argument is not persuasive. Herman clearly and inherently teaches the separation of non-hybridized probes in the Southern blot technique (Example 2 and Figure 1). Moreover, it is well known to an ordinary practitioner skilled in the art that in any hybridization reaction (no matter how specific the target sample is), there is always a molar excess of unhybridized probes, which must be removed by washing and other means to reduce the background signal of hybridization.

> C) Applicant also argues (Page 5, first two lines and page 6, line 3 of third paragraph, and page 8, line 3) that each reference individually does not teach all the elements of the claimed invention. This argument is not persuasive. In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).
>
> D) Applicant also argues (Page 5, lines 3-4 and page 6, lines 5-9 of third paragraph, and page 8, lines 4-8) that there is no motivation to combine the references. This argument is not persuasive, especially in the presence of strong motivation provided by Koster since Koster states, "In addition, because the instant disclosed processes allow the nucleic acid fragments to be identified and detected at the same time by their specific molecular weights (an unambiguous physical standard), the disclosed processes are also much more accurate and reliable than currently available procedure (Column 4, lines 50-55)." Similar logic is applicable to other combinatory references.
>
> In view of the response to arguments, all previous 103(a) rejections are hereby properly maintained.

Applicant respectfully traverses the foregoing rejection.

Claim 1, from which claims 2-5 and 7-23 depend, has been amended herein to more clearly define what applicant regards as the invention. As such, claim 1 now recites "[a] method for identifying cytosine methylation patterns in genomic DNA samples, said method comprising the steps of:

a) chemically treating a genomic DNA sample in such a way that cytosine and 5-methylcytosine react differently and a different base pairing behavior of the two products is obtained in the duplex;

b) enzymatically amplifying portions of the thus-treated DNA sample nonspecifically with regard to methylation of said genomic DNA sample;

c) binding the amplified portions of the thus-treated DNA sample to a surface;

d) contacting a set of probes of different nucleobase sequences, each of which contains the dinucleotide sequence 5'-CpG-3' at least once, to the immobilized DNA samples for hybridization to distinguish methylated and nonmethylated cytosines in said genomic DNA sample;

e) removing any non-hybridized probes from the immobilized DNA samples;

f) analyzing the hybridized probes in a mass spectrometer, wherein the position of the hybridized probes on the surface permits a classification of the immobilized DNA sample hybridized thereto;

g) assigning a peak pattern obtained from the mass spectra to a methylation pattern for the immobilized DNA and comparing the peak pattern with a database to identify cytosine methylation patterns in the genomic DNA sample."

Thus amended, claim 1 is not rendered obvious over Herman et al. in view of Köster for at least the following reasons: First, the foregoing rejection is apparently predicated, at least in part, on the Patent Office's contention that Herman et al. teaches steps (a) through (e) of claim 1. However, such a contention is in error. Herman et al. relates to a technique referred to therein as "methylation specific PCR" or MSP. According to Herman et al., MSP involves "contacting a nucleic acid-containing specimen with an agent that modifies unmethylated cytosine, amplifying the CpG-containing nucleic acid in the specimen by means of CpG-specific oligonucleotide primers, wherein the oligonucleotide primers distinguish between modified methylated and non-methylated nucleic acid and detecting the methylated nucleic acid" (col. 5, line 62 through col. 6, line 1, of Herman et

al.). In other words, MSP "uses the PCR reaction itself to distinguish between modified methylated and unmethylated DNA" (col. 4, line 67 through col. 5, line 1 of Herman et al.) as the "MSP primers themselves are specifically designed to recognize CpG sites to take advantage of the differences in methylation to amplify specific products" (col. 5, lines 6-8, of Herman et al.). Because Herman et al. is predicated upon the preferential or specific amplification of methylated, as opposed to unmethylated, nucleic acids (or vice versa), it follows that Herman et al. cannot involve the amplification of **both** methylated **and** unmethylated fragments.

By contrast, the claimed method does not use PCR to **distinguish** methylated DNA from unmethylated DNA. Instead, the present invention involves amplifying DNA fragments **without regard to methylation**. This means that, pursuant to the claimed invention, **both** methylated and unmethylated fragments are typically amplified. The distinguishing of methylated DNA fragments from unmethylated DNA fragments does not occur in the claimed method during amplification, but rather, occurs after amplification, namely, by hybridizing a set of probes to the amplified fragments.

In other words, whereas Herman et al. involves **using amplification specifically to distinguish between methylated and unmethylated DNA**, the claimed invention involves nonspecifically amplifying **both** methylated and unmethylated fragments and, **thereafter**, hybridizing a set of specific probes to the already amplified DNA to distinguish between methylated and unmethylated DNA.

In view of the above, it can readily be appreciated that steps (b) and (d) of claim 1 are neither taught nor suggested by Herman et al. Because Herman et al. uses PCR to distinguish between methylated and unmethylated DNA, there would be no reason for Herman et al. to hybridize methylation-specific probes to the immobilized amplificates as no additional information would be

imparted by the use of such probes. Similarly, the claimed step (e) of removing any non-hybridized probes from the immobilized DNA samples is neither taught nor suggested by Herman et al. since there would be no reason to perform step (e) in MSP. As noted above, this is because all of the MSP amplificates are inherently indicative of methylation; therefore, there would be no reason to hybridize any probes to the MSP amplificates for the purpose of indicating methylation and there would be no reason to remove any non-hybridized probes.

With regard to the Patent Office's comment in Paragraph A of the "Response to Arguments" that "Herman reference clearly teaches the amplification of both methylated and unmethylated fragments (Column 23, line 67 to Column 24, line 3)," Applicant respectfully submits that the Patent Office has misread the cited passage. The passage in question reads as follows:

> This marked difference in sequence following bisulfite treatment suggested that the method of the invention for specific amplification of either methylated or unmethylated alleles was useful for identification of methylation patterns in a DNA sample.

Contrary to the Patent Office's contention, the foregoing passage does not teach the simultaneous amplification of methylated and unmethylated fragments, but rather, teaches the "**specific** amplification of **either** methylated **or** unmethylated alleles." (Emphasis added.) In other words, this passage teaches using PCR to select for methylated or unmethylated alleles; consequently, the passage is entirely consistent with the distinction drawn above by Applicant.

Applicant also wishes to point out that, with respect to the Patent Office's reference on page 3 of the outstanding Office Action to Example 2 of Herman et al., Example 2 of Herman et al. involves the bisulfite sequencing of a bisulfite treated methylation specific polymerase reaction amplified gene fragment. However, nucleic acid sequencing and methylation specific polymerase

amplification are not involved in the present method. Example 2 of Herman et al. further teaches the methylation sensitive restriction enzyme digest of target DNA followed by Southern Blot of the fragments as a comparison to the use of methylation specific amplification. As the use of Southern blot analysis is used as a comparison to the methylation specific PCR, Herman et al. clearly teaches away from the combination of components of the two methods. The use of components of the Southern Blot method in combination with chemical reagents that distinguish between cytosine and 5-methylcytosine is not taught or suggested nor is it obvious how or why this would be done simply from the fact that the two methods appear in the same reference.

Köster, which relates to a mass spectrometer-based process for detecting a particular nucleic acid sequence in a biological sample, fails to cure the above-noted deficiencies of Herman et al.. Moreover, for reasons already of record, there would have been no motivation for combining the two references.

It should be noted that the present method enables a greater number and variety of CpG positions to be analyzed and is much less constrained by primer design than the Herman method, which only enables those CpG positions covered by the primers to be analyzed. By using the probes of the claimed method, a greater number of CpG positions may be analyzed, and the assay therefore has a much higher versatility than that in which the CpG positions to be investigated are limited by, for example, the formation of primer-dimers, non-specific hybridization and other such considerations that must be taken into account when designing primer mediated nucleic acid amplifications. Moreover, the sequencing of bisulfite treated nucleic acids taught in Example 2 of Herman et al. is a difficult and laborious procedure that has not proven to be suitable for high throughput analysis. The suitability of the claimed method for the analysis of multiple CpG

positions in a fast and cheap methodology suitable for both research and diagnostic laboratories fills a long-felt need for alternative methylation analysis techniques, particularly in the fields of cancer and disease diagnosis and research.

The claims dependent from claim 1 recite additional features and are further patentable over the applied combination of references. For example, with respect to claim 7, Applicant disagrees with the Patent Office's statement on page 3, penultimate paragraph, of the outstanding Office Action that Herman et al. teaches "a method, further characterized in that the immobilized complementary oligonucleotide sequences contain modified bases, ribose or backbone units (Example 2, Figures 1 and 2)." Applicant finds no mention of immobilized oligonucleotide sequences containing modified bases, ribose or backbone units in Example 2 and Figs. 1 and 2.

Claim 8 is further distinguishable over the applied combination of references for the reason that Herman et al. only contemplates a method that allows for methylation-specific amplification of single fragments. While it may be possible to *repeat* this method with different single fragments until a certain percentage of the genome is reached, Herman et al. does not teach or suggest doing so in one step. For example, to reach a coverage of 0.01% of the genome with the method of Herman et al., 1000 different fragments (assuming 300 bp average length) would have to be produced in as many different reactions. Herman et al. does not contemplate the generation of as many as 1000 fragments in one step whereas the present method does.

With respect to claim 9, Applicant disagrees with the Patent Office's comments on page 4, first paragraph, of the outstanding Office Action that Herman et al. teaches "a method, further characterized in that the mixture of amplified DNA fragments is bound to a surface, on which a multiple number of different points is arranged, each of which can bind different portions of the

amplified DNA sample (Figure 1)." Applicant finds no mention of amplified DNA fragments bound to surfaces in Fig. 1 or in the corresponding portion of the specification. The use of the Southern Blot technique does not generally include enzymatic amplification; hence, it would not be equivalent to step d) of the claimed method.

Applicant additionally wishes to point out that, with respect to the Patent Office's comment in the outstanding Office Action that "Herman et al teach a method, further characterized in that a set of probes is used in d), which contains the dinucleotide sequence 5'-CpG-3' only once in each probe and the probes otherwise contain either no cytosine or no guanine bases (Column 18, SEQ ID No:130)" (page 4, second paragraph), the sequence cited by the Patent Office refers to a primer, such as would be used in an amplification step, and not a probe, such as would be used in a distinguishing step.

Accordingly, for at least the above reasons, the foregoing rejection should be withdrawn.

Claim 6 stands rejected under 35 U.S.C. 103(a) "over Herman et al. (U.S. Patent 6,265,171 B1) (July 24, 2001) in view of Koster (U.S. Patent 5,605,798) (February 25, 1997) further in view of Katouzian-Safadi et al. (Biochimie, (1994), Vol. 76, (2), pages 129-132)." In support of the rejection the Patent Office states the following:

> Herman et al. in view of Koster teach the method of claims 1-5, and 7-23 as described above.
>
> Herman et al. in view of Koster do not teach the method, further characterized in that the oligonucleotide bound to the surface contain 5-bromouracil structural units.
>
> Katouzian-Safadi et al. teach the method, further characterized in that the oligonucleotide bound to the surface contain 5-bromouracil structural units (Summary and Results Section).

> It would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to combine and substitute the oligonucleotide containing 5-bromouracil structural units of Katouzian-Safadi et al. in the mass spectrometric method of detection of methylated nucleic acid using agents which modify unmethylated cytosine and distinguish modified methylated and unmethylated nucleic acids of Herman et al in view of Koster since Katouzian-Safadi et al. state, "The substitution of thymine by 5-bromouracil in DNA increases the photocrosslinking yield, and reduces the direct damages to both DNA and protein (Summary, second sentence)." An ordinary practitioner would have been motivated to combine and substitute the oligonucleotide containing 5-bromouracil structural units of Katouzian-Safadi et al. in the mass spectrometric method of detection of methylated nucleic acid using agents which modify unmethylated cytosine and distinguish modified methylated and unmethylated nucleic acids of Herman et al. in view of Koster in order to achieve the express advantage, as noted by Katouzian-Safadi et al., of the substitution of thymine by 5-bromouracil in DNA, which increases the photocrosslinking yield, and reduces the direct damages to both DNA and protein.

Applicant respectfully traverses the foregoing rejection. Claim 6 depends from claim 1. Claim 1 is patentable over the combination of Herman et al. and Köster for at least the reasons set forth in the previous rejection. Katouzian-Safadi et al. fails to cure all of these deficiencies. Consequently, the applied combination of Herman et al., Köster and Katouzian-Safadi et al. does not render obvious claim 6. Moreover, Applicant respectfully submits that one of ordinary skill in the art would not have been motivated to use the teachings of Katouzian-Safadi et al. in the manner proposed by the Patent Office. The alleged advantages recited in Katouzian-Safadi et al. are too general and remote to the present set of facts to have motivated a person of ordinary skill in the art to make the proposed modification.

Accordingly, for at least the above reasons, the foregoing rejection should be withdrawn.

Claim 24 stands rejected under 35 U.S.C. 103(a) "over Herman et al. (U.S. Patent 6,265,171 B1) (July 24, 2001) in view of Koster (U.S. Patent 5,605,798) (February 25, 1997) further in view of Stratagene Catalog (1988, Page 39)." In support of the rejection, the Patent Office states the following:

> Herman et al. in view of Koster expressly teaches the claims 1-5, and 7-24 as described above in detail.
>
> Herman et al. in view of Koster do not teach the motivation to combine all the reagents for identification of cytosine methylation patterns in a genomic DNA samples in the form of a kit.
>
> Stratagene catalog teaches a motivation to combine reagents into kit format (page 39).
>
> It would have been prima facie obvious to one having ordinary skill in the art at the time the invention was made to combine a suitable container, a sample holder for a mass spectrometer, all the reagents for identification of cytosine methylation patterns in a genomic DNA samples, as taught by Herman et al. in view of Koster into a kit format as discussed by Stratagene catalog since the Stratagene catalog teaches a motivation for combining reagents of use in an assay into a kit, "Each kit provides two services: 1) a variety of different reagents have been assembled and pre-mixed specifically for a defined set of experiments. Thus one need not purchase gram quantities of 10 different reagents, each of which is needed in only microgram amounts, when beginning a series of experiments. When one considers all of the unused chemicals that typically accumulate in weighing rooms, desiccators, and freezers, one quickly realizes that it is actually far more expensive for a small number of users to prepare most buffer solutions from the basic reagents. Stratagene provides only the quantities you will actually need, premixed and tested. In actuality, the kit format saves money and resources for everyone by dramatically reducing waste. 2) The other service provided in a kit is quality control". (page 39, column 1).

Applicant respectfully traverses the foregoing rejection. Claim 24 depends from claim 1. Claim 1 is patentable over the combination of Herman et al. and Köster for at least the reasons set

forth above. Stratagene fails to cure all of these deficiencies. Consequently, the applied combination of Herman et al., Köster and Stratagene does not render obvious claim 24. Moreover, Applicant respectfully submits that one of ordinary skill in the art would not have been motivated to use the teachings of Stratagene in the manner proposed by the Patent Office. The alleged advantages recited in Stratagene are too general and remote to the present set of facts to have motivated a person of ordinary skill in the art to make the proposed modification. The Patent Office's argument, taken to its logical endpoint, would render any reagent kit obvious on the basis of Stratagene. This obviously cannot be the correct outcome.

Accordingly, for at least the above reasons, the foregoing rejection should be withdrawn.

In conclusion, it is respectfully submitted that the present application is in condition for allowance. Prompt and favorable action is earnestly solicited.

If there are any fees due in connection with the filing of this paper that are not accounted for, the Examiner is authorized to charge the fees to our Deposit Account No. 11-1755. If a fee is

required for an extension of time under 37 C.F.R. 1.136 that is not accounted for already, such an extension of time is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,

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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Mail Stop Fee Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on April 8, 2004

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